the chamber includes a screen to filter out large components, the proteins may pass through the screen and accumulate in the interstices of the particulate substrate especially if that substrate is of the type described in U.S. Pat. No. 3,505,785 of A.pr. 14, 1970, a substrate which is desirable because each particle is a macroparticle with an impervious core having a coating of monolayers of colloidal microparticles thereby providing a substantial surface area.

A typical source of plugging proteins is serum, i.e. blood from which suspended cellular elements have been removed usually by centrifugation. Thus, if undiluted serum is assayed using an immunoadsorbent of the type described in the above referenced patent and patent applications, the relatively large proteins pass through the filter and tend to plug up the immunoadsorbent. The serum may be diluted to reduce the concentration of proteins for better flow through the immunoadsorbent. Dilution, however, also reduces the concentration of the antigens in the serum. Where the concentration of free antigen in serum is relatively low, e.g. digoxin and T_3, diluting the serum reduces the concentration even more and presents problems in accurately and reliably assaying for the relatively small amounts of antigen present in the diluted serum. Thus, dilution to avoid a plugging problem leads to problems of accurate assays, i.e. the sensitivity of the assay is less than desired.

DESCRIPTION OF THE PRIOR ART

It is known from the literature that antibodies may be isolated by use of immunologic adsorbents, the technique being useful for isolation and purification of antibodies rather than quantitative determination thereof, see *Campbell et al, Proc. Nat'l. Acad. Sci.* U.S. 37 (1951) 575.

The use of an antibody coupled to an insoluble polymer for extracting specific antigens for purposes of isolating and purifying the same is described in Weetall et al, *Biochem. Biophys. Acta.* 107 (1965) 150–152.

Porous glass has been described as a substrate for immobilizing enzymes, see Weetall, *Biochem. Biophys. Acta.* 212 (1970) 1–7. There, glass was treated with gammaaminopropyltriethoxysilane and the isothiocyanate derivative was prepared by treatment with thiophosgene. The enzyme was coupled to the isothiocyanate derivative. Also described in the preparation of an arylamine derivative by the reaction of alkylamine glass with P-nitrobenzoyl chloride followed by use of sodium dithionate to reduce the nitro groups. The arylamine glass was then diazotized and the enzyme coupled thereto.

Weetall, in *Biochem. J.* (1970) 117, 257–261 also describes the use of antibodies bound to porous glass through a silane coupling agent, the immunoadsorbent being used to isolate and purify specific antigens. The data given, however, shows that the reused column, in which the antigen was eluted from the immobilized antibody immunoadsorbent was quite erratic in performance since the recovery of released antigen varied from 74% to 100%. See also U.S. Pat. No. 3,652,761 of Mar. 28, 1972. While useful as an isolation system, the described system has considerable objections from the standpoint of a useable tool in quantitative analysis in which there must be substantially stoichiometric release of the antigen.

U.S. Pat. No. 3,555,143 of Jan. 12, 1971, relates to radioimmunoassay procedures in which an immobilized immunoadsorbent is used only once and then discarded. The immunoadsorbent is a dextran (Sephadix G 25, superfine) cross-linked with glycerine ether bridges and substituted with p-nitrophenoxy-hydroxy-propyl ether groups. The nitro groups are reduced to amine groups using sodium dithionite. The Sephadex substituted with p-amino-phenoxy-hydroxy-propyl groups was then treated with thiophosgene to form Sephadex substituted with p-isothiocyanate-phenoxy-hydroxypropyl groups, the antibody being bound to the latter substituted product.

A reaction widely used to insolubilize a protein involves a covalent binding of the protein to a cyanogen bromide activated cellulose matrix. The mechanism of such activation is set forth in Bartling et al, *Biotechnology and Bioengineering*, Vol XIV (1972) 1039–1044.

U.S. Pat. Nos. 3,502,888 of July 13, 1971; 3,639,559 of Feb. 1, 1972, and 3,720,760 of Mar. 13, 1972, are also of interest.

Where an immobilized immunoadsorbent is to be used only once and discarded, the long term properties of the substrate are not of major consequence. Thus, materials such as Sephadex (dextran) or Sepharose (beaded agarose product) operate satisfactorily as substrates for antibodies bound thereto as described in U.S. Pat. No. 3,555,143, supra.

One of the objections is the tendency of Sephadex and Sepharose type products to dehydrate, that is, the gel collapses and packs to such an extent that flow through the mass is substantially impeded and the availability of antibody for binding antigen is altered, thus effecting the reproducibility and stability of the immunoadsorbent for repeated use.

Glass and other solid inorganic materials offer a desirable alternative because they can be formed into beads to provide better flow and easier packing into a column type arrangement. Such materials do not collapse and are not subject to dehydration during periods of extended use. While a desirable alternate, glass type products also suffer from disadvantages. One of the problems is obtaining a sufficient binding of the antibody to the substrate. Either an insufficient initial binding takes place to provide the activity needed for a quantitative analysis tool, or the activity changes over the life of the immunoadsorbent by undesirable release of antibodies.

Where the glass is highly porous, as that used by the Weetall references cited, there is so much active glass surface area that ample binding of the antibody takes place but non-specific binding of the antigen also takes place. Thus, the antigen bound to the glass is not released completely. That is, rather than having a stoichiometric release, for each use thereof, as is needed for quantitative analysis, the release characteristics are variable and unpredictable. This is confirmed by the Weetall data. Since such glass is usually 96% air or void space, there is considerable active surface area of the glass, not occupied by antibody which serves as an antigen binding site.

Another difficulty with highly porous glass products is that there are multiple crevices in the pores which result in trapping in the crevices and slow release because of the slow diffusion in the crevices. Where a fast response is needed, as for example in automated equipment, the diffusion of the reactants is a rate limiting step and, as is well known, diffusion may be a relatively slow process. Thus, even if not bound to the substrate, the diffusion of the antigen is relatively slow and thus, for the purpose of rapid automated assay equipment, the